

**Shipleigh-Skinner Reserve – Riverside County Endowment:
Diet analyses of pollinator predators in sage scrub and chaparral ecosystems**

Paul Masonick & Dr. Christiane Weirauch
Department of Entomology
University of California, Riverside

RESEARCH AIMS AND SIGNIFICANCE

- (1) Assess the distribution and abundance of four common, flower-visiting, predatory Southern California assassin bug species (Hemiptera: Reduviidae) on their host plants in sage scrub and chaparral habitats of the Shipley-Skinner Reserve.
- (2) Use gut metabarcoding analyses to test the hypothesis that native pollinators constitute a significant proportion of assassin bug diets, and that the diets reflect the pollinator communities found on associated host plants.
- (3) Investigate if predator size affects prey range and abundance of different prey species in the assassin bugs' diet.

Through the documentation of predator-pollinator interactions and associated host plants, we will contribute valuable ecological and molecular information on predators of pollinators, some of which may provide pollination services to rare or important native plants.

BACKGROUND AND RATIONALE

The fitness of many flowering plants depends heavily on the services of pollinating insects; reciprocally many insects, bees in particular, require pollen and nectar from flowers for sustenance.¹ Flowering plants which have evolved mutualistic relationships with specific pollinator species are especially vulnerable to changes in the environment. In many cases, a reduction in the pollinating services provided by a specific insect can adversely affect plant populations.² The presence of predators on flowers has been shown to reduce pollinator flower visitation, causing some pollinators to spend less time at or avoid certain flowers, or potentially diminish populations of specialist pollinators of rare native plants.³⁻⁵ A thorough understanding of pollinator-predator interactions in natural habitats that comprise a diversity of flowering plant species is therefore critical.

Assassin bugs are a diverse (7,000 spp.) family of arthropod predators and blood-feeding insects that include biological control agents and human disease vectors, as well as species that may have negative impacts on beneficial insects such as pollinators.^{6,7} More than half of all assassin bug species are suspected to be associated with the foliage of plants, and many with flowering vegetation where they are thought to hunt for flower-visiting insects including pollinators.⁷⁻¹³ Surprisingly given their abundance in many habitats, prey data for most assassin bug species are unavailable in natural habitats beyond anecdotal observations (e.g., photographs or predator-prey interactions posted online; Fig. 1). In addition to *Apis mellifera* Linnaeus, which appears to be a common prey item among Southern California assassin bugs, native bee species also fall victim to these assassin bugs and may constitute an important component of their diet.

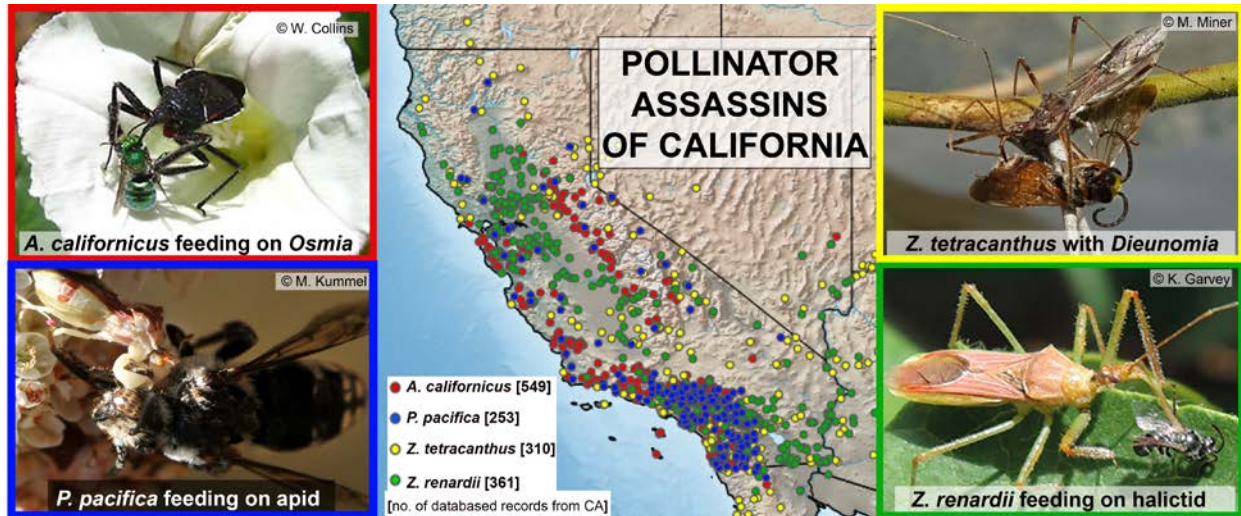


Figure 1. Distribution of target assassin bugs in California and predation on native bees. Number of records for California from the Plant Bug Planetary Biodiversity Inventory (PBI) database (<http://research.amnh.org/pbi/databases/locality>).

Thus, assassin bugs likely have an influence on pollinator communities and therefore the potential to affect plant communities. Given that assassin bugs typically feed on prey organisms within a given size range, we predict that species with different total length may prey on different pollinator species.¹⁴ We here propose to assay the ecological interactions of four common flower-visiting assassin bugs (Fig. 1) that differ in body size and engage in different predatory techniques: *Phymata pacifica* Evans (6.5-7.5 mm), *Zelus renardii* Kolenati (10.5-14.25 mm), *Zelus tetracanthus* Stål (11.3-15.6 mm), and *Apiomerus californicus* Berniker & Szerlip (14.2-16.6 mm).¹⁵⁻¹⁷ The ambush bug *P. pacifica* is most commonly found on *Eriogonum fasciculatum* and a variety of Asteraceae, e.g., *Ericameria* spp., in chaparral habitats. These cryptically colored sit-and-wait predators capture pollinators with raptorial forelegs.¹⁸ The bee killer assassin bug *Apiomerus californicus* is frequently encountered in sage scrub habitats on *Encelia* sp., *Pseudognaphalium californicum*, and *Eriogonum fasciculatum*.¹⁵ Species of *Apiomerus* coat their forelegs with plant resins to ensnare larger prey insects.¹⁹ Leafhopper assassin bugs, *Zelus renardii* and *Z. tetracanthus*, are found on a variety of flowering shrubs and forbs in both environments. *Zelus* spp. use sticky traps for prey capture similar to *Apiomerus*, but produce their own sticky substance from sticky glands on the raptorial legs.¹⁷ The four species are thought to be generalist predators of flower-visiting insects, but no targeted study has so far surveyed any of these four species under natural conditions.²⁰ While data on host association of reduviids with rare native plants in Southern California are lacking, these bugs may still impact rare flowers through food web interactions. Bumble bees, for example, visit many of the same flowers that reduviids frequent and also may provide pollination services to rare plants (Fig. 2).²¹

One of the limitations in studying predator-prey observations is that direct observation for a meaningful sample size is time consuming and often not feasible. Gut assays are now commonly used to study animal diets and predator/prey association when direct field observation is impractical.²² For predators that are suspected to be generalist feeders, like many assassin bug species, metabarcoding approaches employ non-specific primers to amplify DNA found in the gut.²²⁻²⁴ Metabarcoding uses DNA based identification and high-throughput sequencing for

biodiversity assessment. We here propose to use DNA metabarcoding for dietary analyses of the four most commonly encountered Southern California assassin bug species to determine the diversity and abundance of pollinators being preyed upon. We suspect that assassin bug species may exert different levels of predation on different size classes of pollinators and anticipate a correlation between predator size and prey species, with larger assassin bugs consuming a greater proportion of larger pollinators than smaller bugs.

Southern California <i>Bombus</i> spp.						Predators				
<i>B. bifarius</i>	<i>B. centralis</i>	<i>B. crotchii</i>	<i>B. flavifrons</i>	<i>B. fervidus</i>	<i>B. vandykei</i>	HOST PLANTS	<i>A. californicus</i>	<i>P. pacifica</i>	<i>Z. renardii</i>	<i>Z. tetracanthus</i>
				X		<i>Astragalus</i> *				
	X	X	X	X	X	<i>Cirsium</i>	X	X		
						<i>Encelia</i>	X			X
X	X					<i>Ericameria</i>		X		X
					X	<i>Eriodictyon</i>	X			
						<i>Eriogonum</i>	X	X	X	X
X		X			X	<i>Lupinus</i>	X		X	X
	X	X			X	<i>Phacelia</i> *				
X	X		X	X	X	<i>Penstemon</i> *				
		X		X	X	<i>Salvia</i>	X			

Figure 2. Bumble bees from Southern California that pollinate both rare native plants and common host plants of assassin bugs. * denotes rare plants classified by 2013 MSHCP Rare Plant Survey Report.³⁰ Red indicates host to both *Bombus* spp. and Reduviidae. Gray indicates missing host data for Reduviidae. Host plant association from the PBI database (<http://research.amnh.org/pbi/databases/locality>) and [21].

RESEARCH OBJECTIVES

To assess the impact of predators on pollinator communities we will:

- (1) collect assassin bugs and pollinators from the Shipley-Skinner Reserve while documenting their host plant associations.
- (2) document the diversity and abundance of flower-visiting insects both in the field and in assassin bug gut assays to evaluate the relative contribution of native vs. non-native pollinator species to reduviid diets, and determine whether predator size informs prey size.

RESEARCH PLAN

Objective 1: Specimen Collection

Sage scrub and chaparral habitats in the Shipley-Skinner Reserve will be searched in early June for suitable 100m x 100m plots for collecting. Four plots will be established in each of the habitats of interest and will be chosen based on presence of the main host plants associated with the target reduviid species (knowledge of local host associations are based on prior field work in Southern California). Each plot will be visited for one hour once early in the week and then again later in the week for four consecutive weeks or until we have obtained 200 specimens for each of the four target reduviid species. The first visit every week will allow us to determine which plants are being actively used by assassin bugs and will allow us to target our pollinator sampling. Plants which yield reduviids will be flagged and sampled specifically for pollinators during the following visit. To account for any temporal biases in flower visitation by pollinators,

plots that are subjected to a morning sampling will then be searched during an afternoon visit later in the week and vice versa. Assassin bugs will be directly sampled from host vegetation. Cryptically-colored *Phymata* and *Zelus* specimens are best collected by means of beating and sweeping vegetation. *Apiomerus californicus*, which is a large aposematically colored insect, is easily spotted on vegetation and will be sampled by hand-netting. We will also actively sample pollinators from the main plants associated with assassin bugs. All specimens will be preserved in 95% ethanol and kept in a -20C freezer to limit DNA degradation in the Weirauch Lab at the University of California, Riverside (UCR). Observed acts of predation will be recorded in the field, and host associations will be recorded for all reduviids and pollinators collected. To ensure rare plant communities are not damaged by our study, only images will be taken of host plants in the field. Unknown plants from which reduviids are collected will have their GPS coordinates recorded and images later used for identification.

Objective 2: Diet Assays & Size Correlation

DNA-based gut content assays for Reduviidae have been performed in the Weirauch lab in the past, providing unique insights into predator-prey interactions of elusive termite predators and bloodfeeding kissing bugs.^{25,26} In the proposed study, the mid-gut of reduviid specimens collected from the Shipley-Skinner Reserve will be dissected. Based on prior observation, we expect that ~50% of the collected assassin bug specimens will have fed recently enough (gut appears “full”) for further processing, resulting in a sample size of 96 gut samples per assassin bugs species. We will target a short region of the cytochrome oxidase 1 (CO1) standard barcoding region for amplification using an existing set of primers (ZBJ-*ArtF1c* and ZBJ-*ArtR2c*; 157 base pairs) that has been specifically designed to amplify short regions of degraded insect DNA for metabarcoding analyses in gut contents.^{24,27} Blocking primers containing 3' C3-spacers will be used to prevent amplification of non-target (reduviid) DNA. Sequencing will be performed using the Illumina MiSeq platform at the Institute for Integrative Genome Biology (IIGB) at UCR. The resulting sequences will be searched for matches in reference databases (GenBank, iBOL) for identification to pollinator genus, or where feasible, species. All pollinators collected from the field will be mounted and identified. The Entomology Research Museum at UCR has a particularly strong reference collection of native wild bees from California that will be used for comparative identification (www.entmuseum.ucr.edu). The Senior Museum Scientist, Doug Yanega, is a specialist on native Apoidea and will be consulted in difficult cases.

CO1 sequences exist in databases for many of the pollinators native to Southern California that we expect to encounter at the Shipley-Skinner Reserve. In the Barcode of Life Database (BOLD: boldsystems.org), hundreds of sequences are available for North American pollinator species from families that are commonly preyed upon by assassin bugs. However, the majority of these sequences are from specimens collected in Canada and will likely not provide species-level hits for our Southern California-collected specimens. We will build upon the existing reference library to aid in identifications by submitting molecular voucher specimens to BOLD, which offers free barcoding for any local taxa not yet barcoded. Additionally, these sequences will be available for future studies on pollinators found in the Shipley-Skinner Reserve. From a

comparison of preliminary estimates of local pollinator diversity with what is present in the BOLD database, we anticipate around 100 additional species may need to be sequenced.²⁸

This approach will allow us to determine which pollinator species (including rare taxa) are being preyed upon by the four assassin bugs species and whether their abundance in the gut reflects the abundance seen on associated host plants. Given the range in size and predation strategies of the four targeted reduviid species, we will test for correlations between the sizes of predators and their prey. We predict that large reduviids, such as bee killer assassin bugs, prey upon larger pollinators (e.g. *Apis mellifera*), potentially affecting dynamics between native and non-native pollinators. From this, we will be able to detect if preferential feeding on certain pollinator species is occurring at the Shipley-Skinner Reserve.

BUDGET

Personnel

GSR-3 stipend for Paul Masonick (\$3,495/mo. @ 3.68% x 3mo.) \$10,871

Undergraduate research assistant (20 hrs/wk x 10 wks @ \$15/hr) \$3,000

Travel

Fleet Services Vehicle Rental: Compact Truck (\$35.46/day x 10 days) \$355

Molecular Materials & Sequencing

\$15/sample x 384 samples \$5,760

(4 libraries x 96 samples each = 384 samples total)

TOTAL \$19,986

BUDGET NARRATIVE

We request \$19,986 in total funding to perform dietary analyses and elucidate important ecological interactions among flora and fauna native to Southern California. \$10,871 will provide a three month graduate research stipend for the summer of 2016 for Paul Masonick while he manages the project. In addition, \$3,000 will support an undergraduate student, led by Paul, for 50% time for ten weeks to assist with specimen collection, gut extraction, PCR, and next generation sequencing (NGS) preparation. Given the large scale of our project (384 gut samples), we have chosen NGS because it is extremely cost effective when compared to traditional Sanger sequencing.²⁹ \$355 will cover the cost of a rental vehicle from UCR Fleet services for 10 days. We are budgeting \$5760 (\$15 per sample) to cover the costs of PCR reagents, primers, blocking primers, library preparation for NGS, and for a single lane of Illumina MiSeq Sequencing at UCR's IIGB.

REFERENCES

- [1] Tepedino, V.J., 1979. The importance of bees and other insect pollinators in maintaining floral species composition. *Great Basin Naturalist Memoirs*, 3.
- [2] Wilcock, C. & Neiland, R., 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science*, 7(6), pp.270–277.
- [3] Reader, T. et al., 2006. The effects of predation risk from crab spiders on bee foraging behavior. *Behavioral Ecology*, 17(6), pp.933–939.
- [4] Tan, K. et al., 2013. Fearful Foragers: Honey Bees Tune Colony and Individual Foraging to Multi-Predator Presence and Food Quality. *PLOS ONE*, 8(9), p.e75841.
- [5] Elliott, N.B. & Elliott, W.M., 1991. Effect of an Ambush Predator, *Phymata americana* Melin, on Behavior of Insects Visiting *Daucus carota*. *The American Midland Naturalist*, 126(1), pp.198–202.
- [6] Grundy, P.R., 2007. Utilizing the assassin bug, *Pristhesancus plagipennis* (Hemiptera: Reduviidae), as a biological control agent within an integrated pest management programme for *Helicoverpa* spp. (Lepidoptera: Noctuidae) and *Creontiades* spp. (Hemiptera: Miridae) in cotton. *Bulletin of Entomological Research*, 97(3), pp.281–290.
- [7] Weirauch, C. et al., 2014. An Illustrated Identification Key to Assassin Bug Subfamilies and Tribes (Hemiptera: Reduviidae). *Canadian Journal of Arthropod Identification*, 26.
- [8] Balduf, W., 1999. Third annotated list of Phymata prey records (Phymatidae, Hemiptera). , 43(2), pp.74–78.
- [9] Balduf, W.V., 1941. Life History of *Phymata Pennsylvanica Americana* Melin (Phymatidae, Hemiptera). *Annals of the Entomological Society of America*, 34(1), pp.204–214.
- [10] Silva, A.C. da & Gil-Santana, H.R., 2004. Predation of *Apiomerus pilipes* (Fabricius) (Hemiptera, Reduviidae, Harpactorinae, Apiomerini) over Meliponinae bees (Hymenoptera, Apidae), in the State of Amazonas, Brazil. *Revista Brasileira de Zoologia*, 21(4), pp.769–774.
- [11] Costa, J. et al., 2003. The epidemiologic importance of *Triatoma brasiliensis* as a Chagas disease vector in Brazil: a revision of domiciliary captures during 1993-1999. *Memórias do Instituto Oswaldo Cruz*, 98(4), pp.443–449.
- [12] Marques, O.M. et al., 2003. Predação de *Apiomerus lanipes* (Fabricius, 1803) (Hemiptera, Reduviidae) sobre *Apis mellifera* (Linnaeus, 1758), no Estado da Bahia, Brasil.
- [13] Hwang, W.S. & Weirauch, C., 2012. Evolutionary History of Assassin Bugs (Insecta: Hemiptera: Reduviidae): Insights from Divergence Dating and Ancestral State Reconstruction. *PLOS ONE*, 7(9), p.e45523.
- [14] R. Cogni, A.V.L.F., 2002. Influence of prey size on predation success by *Zelus longipes* L. (Het., Reduviidae). *Journal of Applied Entomology*, 126(2-3), pp.74 – 78.
- [15] Berniker, L. & S.L.S., 2011. Revision of the crassipes and pictipes species groups of *Apiomerus* Hahn (Hemiptera: Reduviidae: Harpactorinae). *Zootaxa*, 2949, pp.1–113.
- [16] Evans, J.H., 1931. A Preliminary Revision of the Ambush Bugs of North America, (Hemiptera, Phymatidae). *Annals of the Entomological Society of America*, 24(4), pp.711–738.
- [17] Zhang, J. et al., 2016. Evolution of the assassin's arms: insights from a phylogeny of combined transcriptomic and ribosomal DNA data (Heteroptera: Reduvidae). *Scientific Reports*, 6.
- [18] Mason, L.G., 1986. Free-Loaders, Free-Lancers and Bushwhackers: Sexual Dimorphism

- and Seasonal Changes in Prey-Capture Behavior of Ambush Bugs. *The American Midland Naturalist*, 116(2), pp.323–328.
- [19] Choe, D.-H. & Rust, M.K., 2007. Use of Plant Resin by a Bee Assassin Bug, *Apiomerus flaviventris* (Hemiptera: Reduviidae). *Annals of the Entomological Society of America*, 100(2), pp.320–326.
- [20] Szerlip, S.L. (1980) *Biosystematic revision of the genus Apiomerus (Hemiptera: Reduviidae) in North and Central America*. Ph.D. Dissertation, University of California, Berkeley, 481 pp.
- [21] Williams P. H., Thorp R. W., Richardson L. L., Colla S. R. Bumble bees of North America: An identification guide. Princeton University Press; Princeton, NJ: 2014. 208
- [22] Pompanon, F. et al., 2012. Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, 21(8), pp.1931–1950.
- [23] Deagle, B.E., Kirkwood, R. & Jarman, S.N., 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology*, 18(9), pp.2022–2038.
- [24] Zeale, M.R.K. et al., 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, 11(2), pp.236–244.
- [25] Gordon, E.R.L. & Weirauch, C., 2016. Efficient capture of natural history data reveals prey conservatism of cryptic termite predators. *Molecular Phylogenetics and Evolution*, 94(Pt A), pp.65–73.
- [26] Gordon, E.R.L. & Weirauch, C. in prep
- [27] Leray, M. et al., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, 10, p.34.
- [28] Sidhu, C.S. & Wilson Rankin, E.E., 2014. “Identifying plant-pollinator relationships of the Riverside coastal sage scrub ecosystem for habitat and plant conservation.” Shipley-Skinner Endowment 2014 Annual Progress Report.
- [29] Meier, R. et al., 2016. \$1 DNA barcodes for reconstructing complex phenomes and finding rare species in specimen-rich samples. *Cladistics*, 32(1), pp.100–110.
- [30] MSHCP WRC (2013) Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP). Western Riverside County Regional Conservation Authority.